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Controls of the quantum yield and saturation light of isoprene emission in different-aged aspen leaves

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Abstract

Leaf age alters the balance between the use of end-product of plastidic isoprenoid synthesis pathway, dimethylallyl diphosphate (DMADP), in prenyltransferase reactions leading to synthesis of pigments of photosynthetic machinery and in isoprene synthesis, but the implications of such changes on environmental responses of isoprene emission have not been studied. Because under light-limited conditions, isoprene emission rate is controlled by DMADP pool size (S_{DMADP}), shifts in the share of different processes are expected to particularly strongly alter the light dependency of isoprene emission. We examined light responses of isoprene emission in young fully-expanded, mature and old non-senescent leaves of hybrid aspen (*Populus tremula* x *P. tremuloides*) and estimated in vivo S_{DMADP} and isoprene synthase activity from postillumination isoprene release. Isoprene emission capacity was 1.5-fold larger in mature than in young and old leaves. The initial quantum yield of isoprene emission (α_I) increased by 2.5-fold with increasing leaf age primarily as the result of increasing S_{DMADP}. The saturating light intensity (Q_{I90}) decreased by 2.3-fold with increasing leaf age, and this mainly reflected limited light-dependent increase of S_{DMADP} possibly due to feedback inhibition by DMADP. These major age-dependent changes in the shape of the light response need consideration in modeling canopy isoprene emission.

Keywords

dimethylallyl diphosphate; isoprene synthase; leaf ontogeny; light response curves

Introduction

Fast-growing early-successional tree species, especially when young, form leaves constantly at the top and lose senescent leaves at the bottom of the canopy. Highly dynamic age structure of these canopies can significantly alter the whole canopy physiological activity because of age-dependent differences in leaf physiological potentials (Anten & Werger 1996; Anten & Hirose 1998; Al Afas *et al.* 2005; Niinemets *et al.* 2015). In particular, physiological potentials increase with increasing leaf age in young leaves, level off in mature

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leaves and gradually decrease through leaf aging until induction of senescence that leads to very rapid reductions in leaf physiological potentials, and ultimately to leaf abscission (Garcia-Plazaola *et al.* 2003; Miyazawa *et al.* 2003; Jongebloed *et al.* 2004; Munné-Bosch 2007; Niinemets *et al.* 2012).

Once fully expanded, there is still a significant period of foliage mesophyll differentiation, including cellular expansion and formation of intercellular air spaces and plastid development, before the leaf attains the full physiological capacity. The period of mesophyll differentiation is associated with increases in the contents of ribulose-1,5-bisphophate carboxylase/oxygenase (Rubisco) and rate-limiting proteins of photosynthetic electron transport, and with increases in the contents of leaf photosynthetic pigments, carotenoids and chlorophylls (Shesták 1985a; Shesták 1985b; Niinemets *et al.* 2012; Tosens *et al.* 2012). Isoprene emission in emitting species also increases as the leaf matures, but the emission is characteristically induced somewhat later than positive values of photosynthesis are observed (Harley *et al.* 1994; Monson *et al.* 1994; Wiberley *et al.* 2005; Rasulov *et al.* 2014). In fact, as both the formation of photosynthetic pigments and isoprene rely on the same chloroplastic pool of one of the immediate isoprenoid precursors, dimethylallyl diphosphate (DMADP), there can be a competition between pigment synthesis and isoprene emission in developing leaves that constrains the rate of isoprene emission at given capacity of isoprene synthase reaction (Rasulov *et al.* 2014).

In mature leaves, there is a significant turnover of components of photosynthetic machinery, including photosynthetic pigments (Rundle & Zielinski 1991; Demmig-Adams & Adams 1993; Bertrand & Schoefs 1999; Beisel *et al.* 2010). Thus, even in fully-developed leaves, a certain substrate-level competition between pigment synthesis and isoprene emission can still be present, although it is operating at a low to moderate level because in mature leaves, the DMADP flux to larger isoprenoid synthesis is commonly much less than the flux going to isoprene formation (Ghirardo *et al.* 2014; Rasulov *et al.* 2014; Rasulov *et al.* 2015b). However, such a competition becomes increasingly unlikely with increasing leaf age as leaf physiological activity decreases. In older leaves, the rate of replacement of damaged proteins and pigments is expected to decrease because the nitrogen resorbed from non-functional proteins can be increasingly used to support the growth of new leaves or stored in woody tissues to support the growth of foliage in the next growing season.

In modeling isoprene emission, constant light and temperature responses are often used, and only the emission capacity is considered as a leaf-dependent parameter (Guenther *et al.* 1993; Guenther 1997; Monson *et al.* 2012; Grote *et al.* 2013). However, as DMADP pool size importantly controls responses of isoprene emission to environmental variables (Rasulov *et al.* 2009b; Rasulov *et al.* 2010; Li & Sharkey 2013b; Niinemets & Sun 2015), variation in the importance of substrate-level competition through leaf ontogeny can significantly modify the environmental responses of isoprene emission. The asymptotic light response of isoprene emission can be described by three parameters: the initial quantum yield, the light-saturated emission rate, the emission capacity (I_{max}), and the quantum flux density (Q) corresponding to I_{max} (saturation Q). While in the case of net assimilation rate, the initial quantum yield is relatively invariable and does not depend on photosynthetic capacity (Ehleringer & Björkman 1977), the quantum yield for isoprene emission is much

more variable and is often correlated with I_{max} (Monson *et al.* 1992; Harley *et al.* 1996; Harley *et al.* 1997; Harley *et al.* 2004; Sun *et al.* 2012b; Rasulov *et al.* 2015a) due to reasons not yet fully understood.

Obviously, the share of ATP and NADPH produced in light among photosynthetic carbon metabolism and isoprenoid synthesis depends on the overall capacity of chloroplastic 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP pathway) of isoprenoid synthesis. However, once produced, the availability of DMADP for isoprene synthesis can depend on the capacity of its concurrent use in larger isoprenoid synthesis. Given that the Michaelis-Menten constant for DMADP of isoprene synthase is much larger than that for prenyltransferases, in particular, that of geranyl diphosphate synthases, the key enzymes responsible for the initial step of synthesis of larger isoprenoids (Orlova *et al.* 2009; Rajabi Memari *et al.* 2013; Rasulov *et al.* 2014), the enzymatic competition for DMADP by prenyltransferases and isoprene synthase is unequal. In particular, prenyltransferases could significantly draw down DMADP pool size in low light when the rate of DMADP synthesis is small and thereby reduce the rate of isoprene synthesis. Thus, a competition for DMADP among different DMADP-consuming reactions, might significantly alter the initial quantum yield for isoprene emission. With increasing the light level, DMADP becomes increasingly available, and the effect of such a competition on isoprene emission likely becomes gradually less. However, the competition could still shift the light-saturation point of isoprene emission, depending on how large the DMADP pool needs to become to saturate the prenyltransferase reactions, and also on the capacity of isoprene synthase relative to DMADP pool size.

On the other hand, it has been recently demonstrated that accumulation of DMADP can inhibit the overall flux through the MEP/DOXP pathway due to inhibition of deoxyxylulose 5-phosphate synthase, the first enzyme in the pathway (Banerjee *et al.* 2013; Ghirardo *et al.* 2014; Wright *et al.* 2014). Such a feedback inhibition could imply that rising DMADP pool size due to reduction of DMADP use in prenyltransferase reactions or with increasing light availability can inhibit the whole pathway flux, especially when isoprene synthase activity is limited as can occur in older leaves.

Here we studied light responses of isoprene emission in different-aged hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx.) leaves to test the hypothesis that age-dependent variations in DMADP pool size lead to changes in the initial quantum yield and light saturation of isoprene emission. In particular, because the consumption of DMADP for pigment synthesis is greater in young leaves, we hypothesized that (1) DMADP pool size limits the low-light isoprene emission more in young than in mature and old leaves, and that (2) greater light intensities are needed for saturation of isoprene emission rate in young and mature leaves than in old leaves. The results of this analysis demonstrate important variations in the initial quantum yield, emission capacity and saturation light for isoprene emission through leaf development, and suggest that age-dependent differences in DMADP available for isoprene emission play a major role in changes in the shape of the light response curve of isoprene emission.

Material and methods

Plant material

Two-year-old hybrid aspen (*Populus tremula* L. x. *P. tremuloides* Michx.) clone H200 (Rasulov *et al.* 2009a; Sun *et al.* 2012b; Niinemets & Sun 2015 for further information about the genotype) plants were used for the experiments. The saplings were planted in 4 L plastic pots filled with commercial garden soil including slow release NPK fertilizer with microelements (Biolan Oy, Finland) and grown in a Percival growth chamber (CLF Plant Climatics GmbH, Wertingen, Germany) at day/night temperatures of 25/20 °C for 14 h photoperiod and at a quantum flux density of 500 μmol m^{-2} s^{-1}. The relative humidity was between 60-65% and the ambient CO_2 concentration between 380-400 μmol mol^{-1}.

By the time of the measurements, the plants had 20-24 leaves on the main stem. We used fully-expanded young (leaves 4-5 from the top, ca. 12 days old), young fully mature (leaves 10-12 from the top, ca. 30 days old) and old non-senescent fully mature (leaves 18-20 from the top, ca. 60 days old) leaves for these experiments.

Isoprene emission and photosynthesis measurements

We used a Walz GFS-3000 gas exchange system (Walz GmbH, Effeltrich, Germany) together with a proton transfer reaction quadrupole mass spectrometer (PTR-QMS, high sensitivity version, Ionicon, Innsbruck, Austria) for simultaneous measurements of foliage gas exchange and isoprene emission rates. The PTR-QMS system was calibrated frequently with a standard gas containing 4.47 ppm isoprene in N_2 (Hills-Scientific, Boulder, CO, USA).

The measurements were conducted with attached leaves. After enclosure of the leaf in the cuvette, it was stabilized under the baseline conditions of photosynthetic quantum flux density (Q) of 500 μmol m^{-2} s^{-1} (growth light), leaf temperature of 30 °C, chamber humidity of 60%, and CO_2 concentration of 380 μmol mol^{-1} until stomata opened and steady-state rates of net assimilation and isoprene emission were achieved, typically in 20 min. after leaf enclosure. Upon reaching the steady state, isoprene emission and net assimilation rates were recorded under the baseline conditions and dark release of isoprene emission was estimated (see the next section). Thereafter, the leaf was stabilized again in the baseline conditions, and Q (μmol m^{-2} s^{-1}) was changed in the sequence of: 1850 → 0 (measurement of dark decay kinetics) → 1500→1000→ 750→ 500→ 250→ 0 (measurements of dark decay kinetics) → 100→ 50→ 20→ 0. At each new light level (except for transfer to darkness for dark decay kinetics measurements), isoprene emission and foliage gas exchange rates were recorded after a new steady state was established, characteristically in ca. 10 min. following the change of the light level. After the dark decay kinetics measurements (500 → 0, 1850 → 0, 250→ 0) and switching on the light, the leaf was stabilized at the new light intensity until a new steady state had been reached, typically for 20 min after switching on the light again. Leaf net assimilation rate (A), stomatal conductance and intercellular CO_2 concentration (C_i) were computed according to von Caemmerer and Farquhar (1981), and leaf isoprene emission rate according to Niinemets et. al. (2011).

Estimation of in vivo dimethylallyl diphosphate (DMADP) pool size and isoprene synthase rate constant (k)

In vivo pool of DMADP responsible for isoprene synthesis and isoprene synthase rate constant were estimated according to the method of Rasulov et al. (2009a; 2010; 2011). This method is based on the assumption that the initial postillumination release of isoprene emission for 150-200 s after switching off the light relies on DMADP synthesized prior to switching off the light (Rasulov *et al.* 2009a; Li *et al.* 2011; Rasulov *et al.* 2011). Accordingly, the integral of isoprene emission during the initial postillumination burst provides the DMADP pool size corresponding to the steady-state isoprene emission rate in light (Rasulov *et al.* 2009a; Li *et al.* 2011; Rasulov *et al.* 2011). The postillumination isoprene emission burst can rely to some extent on isopentenyl diphosphate that is in equilibrium with DMADP (Li *et al.* 2011; Rasulov *et al.* 2011), but comparisons of the in vivo method and destructive chemical estimations of DMADP pool size have demonstrate a good correspondence between different methods (Rasulov *et al.* 2009a; Weise *et al.* 2013). As denoted in the previous section, dark decay kinetics corresponding to three different light intensities were taken during I vs. Q response curve measurements, and DMADP pool size was estimated in each case. Prior to integration, the decay kinetics were corrected for the leaf chamber effect as in Sun et al. (2012b). The leaf chamber effect was generally less than 10% of estimated DMADP pool size.

Given that the consumption of DMADP during postillumination isoprene release leads both to reduced DMADP pool size and isoprene emission rate, paired values of isoprene emission rate at any given moment of time t, $I(t)$, and DMADP pool size supporting this rate, $S_{\mathrm{DMADP}}(t)$, can be obtained. $S_{\mathrm{DMADP}}(t)$ is given as the integral of isoprene emission rate from time t to the baseline value when all DMADP existing prior to switching off the light and remaining until time t has been consumed. Analogous integration for any value of t through the postillumination decay kinetics provides the kinetic curve of in vivo isoprene synthase. We define the initial slope of this curve as the rate constant of isoprene synthase (k, s^{-1}) (Rasulov *et al.* 2011; Rasulov *et al.* 2015a).

Data analysis

Inverse modeling was used to calculate the rate of photosynthetic electron transport, J, needed to support the given rate of net assimilation (Farquhar & Sharkey 1982; Brooks & Farquhar 1985; Niinemets *et al.* 2002):

$$J=\frac{(A+R_{\mathrm{d}})\,(4C_{\mathrm{i}}+8\Gamma*)}{C_{\mathrm{i}}-\Gamma*}, \quad (1)$$

where C_{i} is the intercellular CO_2 concentration, R_{d} is the dark respiration rate and Γ* is the CO_2 compensation point in the absence of R_{d} (Laisk 1977) calculated according to Niinemets and Tenhunen (1997). Due to the existence of alternative electron sinks and lower CO_2 concentration in the chloroplasts than in the intercellular air space, the actual photosynthetic electron transport rate can be larger than that calculated by Eq. 1. Nevertheless, compared with A, J estimated by Eq. 1 provides a measure of foliage

photosynthetic activity that is independent of possible differences in stomatal openness. Inverse modeling was also used to calculate the minimum estimate of the apparent maximum carboxylase activity of Rubisco (V_{cmax}) that is needed to explain the measured light-saturated net assimilation rate (Niinemets & Tenhunen 1997; Niinemets *et al.* 1999a). Using these estimates of J and V_{cmax}, and Farquhar et al. (1980) photosynthesis model, the relative limitation of photosynthesis at light saturation, Λ_S (%) was calculated as 100[1-$A(C_i)/A(C_a)$], where $A(C_i)$ is the light-saturated net assimilation rate at the measured intercellular CO_2 concentration, and $A(C_a)$ the potential net assimilation rate when C_i equals the ambient CO_2 concentration.

Dependencies of the rates of isoprene emission, net assimilation and photosynthetic electron transport on incident quantum flux density (Q) were fitted by the Smith equation (Niinemets & Tenhunen 1997):

$$y=\frac{\alpha Q}{\left[1+\frac{\alpha^2 Q^2}{y_{max}}\right]^{\frac{1}{2}}}-y_0, \quad (2)$$

where α is the initial quantum yield (α_I for isoprene emission, α_A for net assimilation and α_J for photosynthetic electron transport), and y_{max} is the capacity of the given process (I_{max} for isoprene emission, A_{max} for net assimilation and J_{max} for photosynthetic electron transport), and y_0 is the rate in darkness (y_0 = 0 for isoprene emission and photosynthetic electron transport and $y_0 = R_d$ for net assimilation). Iterative minimization of the sum of error squares between measured and predicted values was used for data fitting.

Equation 2 predicts an asymptotic increase of the process rate with increasing Q, whereas for some combination of model parameters, the saturation Q can be predicted to occur at unrealistically high quantum flux densities (Leith & Reynolds 1987; Causton & Dale 1990). Thus, we define the saturating quantum flux density as the value of Q that is necessary to achieve 90% of the process rate at the quantum flux density of 2000 μmol m^{-2} s^{-1} (Q_{90}). For isoprene emission, Q_{90} is given as:

$$Q_{I90}=\frac{0.9I_{2000}}{\left[\alpha_I{}^2-\frac{\left(0.9I_{2000}\alpha_I\right)^2}{I_{max}{}^2}\right]^{\frac{1}{2}}}, \quad (3)$$

where I_{2000} is the rate of isoprene emission at Q = 2000 μmol m^{-2} s^{-1}. Q_{90} is given analogously for the photosynthetic electron transport rate (Q_{J90}). For the net assimilation rate, Q_{90} equals:

$$Q_{A90}=\frac{0.9A_{2000}+R_d}{\left[\alpha_A{}^2-\frac{(0.9A_{2000}+R_d)^2\alpha_A{}^2}{A_{max}{}^2}\right]^{\frac{1}{2}}}, \quad (4)$$

where A_{2000} is the net assimilation rate at Q = 2000 μmol m^{-2} s^{-1}. To further characterize the rate of asymptotic light saturation of the given process rate, the ratios of physiological capacities (Eq. 2, I_{max}, A_{max}, J_{max}) to the process rates at Q = 1000 μmol m^{-2} s^{-1} (I_{1000}, A_{1000}, J_{1000}) were also calculated.

To compare the light response curves with different I_{max} values, a modified Smith equation was used (Guenther *et al.* 1993; Monson *et al.* 2012). All emission rates were standardized with respect to I at Q = 1000 μmol m^{-2} s^{-1} (I_{1000}) and the data were fitted again by a least squares method.

As DMADP pool size (S_{DMADP}) estimates were available for three different Q values, we calculated the normalized light-dependent change of S_{DMADP} (%) as:

$$\Delta_S = \frac{100\,[S_{DMADP}(Q_2) - S_{DMADP}(Q_1)]}{(Q_2 - Q_1)\left(\frac{S_{DMADP}(Q_2)+S_{DMADP}(Q_1)}{2}\right)}, \quad (5)$$

where $S_{DMADP}(Q_1)$ is the DMADP pool size at the light intensity of Q_1 and $S_{DMADP}(Q_2)$ that at the light intensity of Q_2. The pool size change was normalized with respect to the average pool size to directly compare leaves with different absolute DMADP pool sizes.

The characteristics among leaf ages were compared by separate samples *t*-tests. The saturation light intensities (Q_{90}) for different physiological processes (isoprene emission, net assimilation and photosynthetic electron transport) and the ratios of process capacities to the process rate at Q = 1000 μmol m^{-2} s^{-1} (I_{max}/I_{1000}, A_{max}/A_{1000}, J_{max}/J_{1000}) were compared among different leaf ages by paired samples *t*-tests. For each leaf age class, six replicate measurements were conducted with different plants.

Results

Basic structural and physiological differences among leaves of different age

All leaves included in the analysis were fully expanded with similar size, but young leaves had a lower leaf dry mass per unit area than mature and old leaves (Table 1). Incomplete foliage structural differentiation was also associated with lower capacities for isoprene emission, net assimilation rate and photosynthetic electron transport in young leaves compared with those characteristics in mature leaves (Table 1). However, these capacities were similar among young and old leaves (Table 1). Both intercellular CO_2 concentration and relative stomatal limitation of photosynthesis were independent of leaf age (Table 1). Dark respiration rate was higher in young than in old leaves, and similar among young and mature and mature and old leaves (Table 1).

Leaf age effects on initial quantum yields and saturation light

The initial quantum yield for isoprene emission (α_I) increased with increasing leaf age (Table 1 for average values and Fig. 1 for sample light response curves and for standardized light response curves including all data for given leaf age class). Quantum yields for net assimilation rate (α_A) and photosynthetic electron transport rate (α_J) were independent of

leaf age (Table 1). The saturation light (Q_{90}) for isoprene emission was lower in old than in young and mature leaves (Table 1, Fig. 1), while the only significant contrasts among Q_{90} values for net assimilation and photosynthetic electron transport rates (Q_{A90} and Q_{J90}) were greater Q_{A90} and Q_{J90} values in mature than in old leaves (Table 1). Age differences in the ratios I_{max}/I_{1000}, A_{max}/A_{1000} and J_{max}/J_{1000} typically reflected differences in corresponding Q_{90} values (Table 1).

Controls on isoprene emission rate by isoprene synthase activity and DMADP pool size in different-aged leaves

Differences in isoprene emission rate (I) among leaf ages (Fig. 2a) can result from differences either in DMADP pool size (S_{DMADP}) or in isoprene synthase rate constant (k) or from differences in both controlling factors. Average DMADP pool size increased with increasing leaf age (Fig. 2b), but the isoprene synthase rate constant was similar among young and mature leaves and lower in old leaves (Fig. 3c). Despite higher isoprene synthase rate constant, in lower light, isoprene emission rate was lower in young than in old leaves (Fig. 2a), suggesting that S_{DMADP} more strongly controlled the emission at limiting light. In contrast, at higher light, a similar average rate of isoprene emission for young and old leaves (Fig. 2a) resulted from different combinations of S_{DMADP} and k in young and old leaves (Fig. 2b, c).

Initial quantum yield and saturating quantum flux density for isoprene emission as dependent on DMADP pool size and isoprene synthase rate constant

Across different leaves, α_I increased with increasing S_{DMADP}, but this relationship leveled off and α_I even tended to decrease with increasing S_{DMADP} in old leaves (Fig. 3a). No clear relationship between α_I and k was observed (Fig. 3b). Isoprene emission rate at high light increased with increasing S_{DMADP} in young and mature leaves, but not in old leaves (Fig. 3c). The emission rate was positively correlated with k, but the increase was less for young leaves with lower S_{DMADP} than in mature and old leaves with higher S_{DMADP} (Fig. 3d).

Across all leaves, the saturating quantum flux density (Q_{I90}, Eq. 3) was negatively correlated with S_{DMADP} (Fig. 4a) and positively with k (Fig. 4b) and with the light-dependent increase of DMADP pool size (Eq. 5, Fig. 4c). Q_{I90} and α_I were negatively correlated (Fig. 5a), while Q_{I90} and isoprene emission capacity (I_{max}, Eq. 2) were positively correlated (Fig. 5b). However, α_I and I_{max} were positively correlated, except for old leaves (inset in Fig. 5b).

Correlations among foliage isoprene emission and photosynthetic characteristics through leaf development

Across leaves with different ages, both the quantum yields for net assimilation (α_A) and photosynthetic electron transport (α_J) and corresponding capacities (A_{max} and J_{max}) were strongly correlated ($r^2 = 0.78$ for the quantum yields and $r^2 = 0.72$ for the capacities, $P < 0.001$ for both). The quantum yield for isoprene emission (α_I) did not correlate with either α_A ($r^2 = 0.08$) or α_J ($r^2 = 0.09$, $P > 0.2$ for both), while the capacity for isoprene emission was positively correlated with J_{max} (Fig. 6a). Due to contrasting scaling of quantum yields for isoprene emission, net assimilation and photosynthetic electron transport with leaf age, the ratios of quantum yields, α_I/α_A and α_I/α_J increased with increasing leaf age (Table 1).

The saturating light for isoprene emission was positively correlated with that for net assimilation (Fig. 6b) and photosynthetic electron transport (Fig. 6c). However, for young and mature leaves (Fig. 6b, Table 1), Q_{I90} was larger than Q_{A90} (for comparison of average values, $P < 0.01$ for young and $P < 0.005$ for mature leaves) and Q_{J90} ($P < 0.001$ for young and $P < 0.01$ for mature leaves). For old leaves, Q_{I90} did not differ significantly from Q_{A90} ($P > 0.1$) and Q_{J90} ($P > 0.2$).

Discussion

Leaf age effects on basic foliage physiological characteristics

Foliage expansion growth is typically completed earlier than foliage structural and physiological maturation (Miyazawa *et al.* 2003; Niinemets *et al.* 2012) as was also observed in our study (Table 1). While leaf size did not differ among young and mature leaves studied, young leaves had a lower dry mass per unit area and lower photosynthetic and isoprene emission capacities (Table 1), reflecting their incomplete structural and physiological maturation (s. Introduction for developmental changes during leaf ontogeny).

Although we have investigated non-senescent leaves, there typically is a certain reduction of foliage physiological activity with leaf aging before the rapid onset of decline of foliage physiological function after senescence has been triggered (Harley *et al.* 1994; Niinemets *et al.* 2004; Grassi & Magnani 2005; Sun *et al.* 2012a). This age-dependent decline in foliage physiological activity of non-senescent leaves is associated with remobilization of limiting nutrients such as nitrogen to support the growth of new leaves, especially in fast-growing canopies such as in young aspen plants (Noormets *et al.* 1996; Kull *et al.* 1998; Niinemets *et al.* 2015). A reduction in foliage photosynthetic and isoprene emission capacities in old leaves was also observed in our study such that young and old leaves had similar physiological capacities, ca. 0.5-0.8-fold less compared with mature leaves (Table 1, Fig. 1, 2a). Given that both leaf development and aging comprise significant periods of leaf life span, we argue that these differences play an important role in determining the whole canopy isoprene emission and carbon gain rates in fast-growing canopies of early-successional isoprene emitters such as woody *Populus* and *Salix canopies* or herbaceous *Phragmites* canopy (Niinemets *et al.* 2015 for a discussion of canopy development in dependence on plant functional type and successional status).

Controls of isoprene emission by DMADP pool size and isoprene synthase activity in relation to leaf age

In mature leaves, leaf-to-leaf variation in high-light isoprene emission rate is typically dependent on differences in isoprene synthase activity (Brüggemann & Schnitzler 2002a; Brüggemann & Schnitzler 2002b; Magel *et al.* 2006; Rasulov *et al.* 2010; Rasulov *et al.* 2011; Sun *et al.* 2012b) characterized in our study by the isoprene synthase rate constant (k). However, variation of the isoprene synthase substrate, dimethylallyl diphosphate, DMADP, pool size (S_{DMADP}) can also alter the isoprene emission rate among leaves with given isoprene synthase activity (Sun *et al.* 2012b; Ghirardo *et al.* 2014; Rasulov *et al.* 2014). A control by S_{DMADP} or a shared control by S_{DMADP} and k is expected especially in conditions when isoprene synthase activity is high relative to S_{DMADP} such that isoprene

synthase operates much below its potential capacity, or in conditions when accumulation of S_{DMADP} can lead to a feedback inhibition of MEP/DOXP pathway (s. Introduction and Banerjee *et al.* 2013; Wright *et al.* 2014). In vitro studies further suggest that under extremely high concentrations of dimethylallyl diphosphate, isoprene synthase could be directly inhibited by S_{DMADP} (Silver & Fall 1991; Schnitzler *et al.* 2005), possibly due to inhibition of diphosphate release from the enzyme-product complex or inhibition of active site closure of substrate-enzyme complex (Köksal *et al.* 2010). The latter inhibition has been found at dimethylallyl diphosphate concentrations exceeding ca. 8 mM (Silver & Fall 1991; Schnitzler *et al.* 2005), i.e. at much greater concentrations than observed here (at most ca. 0.25 mM according to the conversion factors based on leaf structural characteristics derived in Rasulov *et al.* 2009a; Rasulov *et al.* 2014).

In our study, DMADP pool size increased with increasing leaf age (Fig. 2b), but the isoprene synthase rate constant was similar among young and mature leaves and lower in old leaves (Fig. 2c). Analysis of correlations of the light-saturated isoprene emission rate with k and S_{DMADP} suggested that a similar light-saturated isoprene emission rate in young and old leaves (Fig. 2a) was achieved in completely different ways. Although the isoprene emission rate increased with increasing k in all cases, at a given k, the emission rate was greater in mature and old leaves (Fig. 3d). Thus, S_{DMADP} limited high-light isoprene emission rate more in young leaves, while in old leaves, S_{DMADP} was vastly over-dimensioned relative to k, and isoprene synthase seemed to operate in DMADP-saturated conditions (Fig. 3c).

Despite very high S_{DMADP} values were observed in the current study in old leaves (Fig. 2b, Fig. 3c), the evidence of a feedback inhibition of isoprene emission by S_{DMADP} in high light was somewhat limited. Indeed, the light-dependent increase of S_{DMADP} was weaker in old leaves than in young and mature leaves, and it was even occasionally close to zero or slightly negative (Fig. 4c), consistent with the feedback inhibition. Nevertheless, in most cases, a lower isoprene emission rate in old leaves was primarily the result of reduced isoprene synthase rate constant, i.e., DMADP supply exceeded its consumption by isoprene synthase. The mismatch between DMADP supply and its consumption leading to a greater DMADP pool size does not rule out the possibility of a feedback control, but it suggests that such a control likely becomes operational at relatively large DMADP concentrations. Similarly to these observations, a previous study demonstrated that very high S_{DMADP} pools in bisphosphonate-inhibited leaves led to only moderate feedback inhibition of MEP/DOXP pathway in hybrid aspen (Rasulov *et al.* 2015b).

What can be responsible for the large pool of DMADP in old leaves? DMADP pool size at any moment of time is the outcome of its production and consumption by prenyltransferases and isoprene synthase. Accordingly, a rise in the DMADP level suggests a certain imbalance between the activity of the MEP/DOXP pathway and DMADP consumption. Such an imbalance is consistent with the hypothesis that DMADP use in constitutive prenyltransferase reactions responsible for photosynthetic pigment synthesis becomes gradually inhibited with leaf aging. It is, however, unclear why MEP/DOXP pathway activity is not concurrently reduced to maintain a certain DMADP pool size in older leaves. Previous studies comparing isoprene-emitting and non-emitting species have suggested that isoprene emission keeps the MEP/DOXP pathway active and allows for a rapid induction of

larger isoprenoid synthesis when needed, e.g., in stressed leaves (Rosenstiel *et al.* 2004; Owen & Peñuelas 2005; Fineschi *et al.* 2013). Analogously, in fully mature leaves where the constitutive prenyltransferase activity is reduced, a greater DMADP pool size could serve to rapidly respond to changes in the requirement for larger isoprenoids in fluctuating environments.

Furthermore, there is evidence that the rate of different processes does decrease with different time kinetics in aging leaves (Harley *et al.* 1994; Monson *et al.* 1994; Andersson *et al.* 2004; Niinemets *et al.* 2004; Keskitalo *et al.* 2005; Sun *et al.* 2012a). In particular, a decrease in foliage photosynthetic activity seems to precede reductions in foliage pigment content (Valjakka *et al.* 1999; Takeuchi *et al.* 2002; Niinemets *et al.* 2004) and isoprene emission capacity (Harley *et al.* 1994; Monson *et al.* 1994; Sun *et al.* 2012a). As a decrease in photosynthetic capacity can led to enhanced production of reactive oxygen species (ROS) due to imbalanced light energy interception and consumption (Huner *et al.* 1998; Foyer *et al.* 2012), maintenance of isoprene emission capacity in aging leaves could importantly contribute to quenching of ROS and preservation of the integrity of cellular metabolism that is necessary for safe dismantling of cellular structures upon senescence (Andersson *et al.* 2004; Keskitalo *et al.* 2005).

Changes in the initial quantum yield for isoprene emission in different-aged leaves

The light-limited isoprene emission rate is typically determined by DMADP pool size (Rasulov *et al.* 2009b; Li & Sharkey 2013a), and thus, any variation in S_{DMADP} in low light should be associated with changes in the initial quantum yield of isoprene emission, α_I. Indeed, in our study, leaf-age dependent variations in S_{DMADP} (Fig. 2b, Table 1) were accompanied with major changes in α_I that was the greatest in old leaves with the largest S_{DMADP}, followed by mature and young leaves (Table 1, Fig. 1).

To our knowledge, such age-dependent variations in α_I, 2.5-fold among old and young leaves (Table 1), and more than 5-fold across all leaves (Fig. 3a), and the connection of these changes to modifications in S_{DMADP} have not been demonstrated so far. However, an analogous difference in α_I was observed in aspen grown under elevated and ambient atmospheric [CO_2] (Sun *et al.* 2012b), and in aspen grown under ambient and high temperatures (Rasulov *et al.* 2015a). In both studies, a lower α_I in elevated-[CO_2]-grown than in ambient-[CO_2]-grown plants and in high-temperature-grown than in ambient-temperature-grown plants was associated with a lower S_{DMADP} (although measured at high light in these studies). These previous observations together with the results of the current study underscore the important role of S_{DMADP} in determining the initial quantum yield of isoprene emission.

Differently from young and mature leaves, the correlation between α_I and S_{DMADP} leveled off and even tended to be negative in old leaves (Fig. 3a). As discussed above, the decline of α_I at higher S_{DMADP} could be interpreted as evidence of a feedback inhibition. However, given that S_{DMADP} did increase with further increases in light level in old leaves, such a reverse trend in α_I more likely reflects the low isoprene synthase rate constant in old leaves (Fig. 3b), again emphasizing the importance of consideration of both the isoprene synthase

activity and S_{DMADP} to predict variations in isoprene emission rate across leaves with different MEP/DOXP pathway and isoprene synthase activities.

How saturating quantum flux density varies with leaf age

At current ambient CO_2 concentrations, light-saturation of foliage net assimilation (Q_{A90}) rate occurs when the limitation of ribulose-1,5-bisphosphate (RuBP) carboxylation due to RuBP regeneration (photosynthetic electron transport limitation) crosses over to Rubisco (CO_2) limitation. As electrons can be used to support both CO_2 fixation and photorespiration and also alternative processes such as nitrate reduction, isoprene synthesis and cyclic and pseudocyclic electron flow (Stitt 1986; Bloom *et al.* 1989; Laisk *et al.* 2007; Laisk *et al.* 2010), photosynthetic electron transport rate typically saturates at higher quantum flux densities (Q_{J90}) than net assimilation rate as was also confirmed in our study (Table 1). On the other hand, isoprene emission rate is more strongly related to the rate of photosynthetic electron transport than to the rate of net assimilation through the light response curve (Niinemets *et al.* 1999b; Rasulov *et al.* 2009b; Morfopoulos *et al.* 2014). In our study, the light-saturation of isoprene emission (Q_{I90}) tended to occur at higher light than that for photosynthetic electron transport, but we emphasize that the electron transport rate estimated here by Eq. 1 is the lowest estimate needed to explain the observed rate of gross CO_2 assimilation and photorespiration and the true rate could be higher (Stitt 1986; Bloom *et al.* 1989).

In our study, a greater Q_{I90} was observed for young and mature leaves, but not for old leaves (Table 1, Fig. 6b, c). In fact, in old leaves, Q_{I90} did not differ from Q_{A90} and Q_{J90} (Fig. 6b, c). Provided that isoprene synthase activity is independent of measurement light intensity (Rasulov *et al.* 2009b, Fig. 2c), for a given leaf, light-dependent increases of isoprene emission and saturation level should be primarily determined by the effects of light on DMADP pool size. Thus, as soon as additional DMADP becomes available with increasing light level, isoprene emission increases until the rate of DMADP production saturates due to reaching the maximum enzymatic capacities of MEP/DOXP pathway or due to limited supply or NADPH and/or ATP or due to feedback inhibition by DMADP. Given that the light-saturation of net assimilation rate is driven by the limited concentration of CO_2 at carboxylation sites, a greater fraction of NADPH and ATP becomes available for DMADP formation at higher light, explaining the greater saturating light for isoprene emission than that for net assimilation.

Due to strong correlative patterns among the drivers of isoprene emission rate at different light intensities, a causal explanation of Q_{I90} variation across leaves of different age is somewhat complicated. First, Q_{A90} and Q_{J90} were also lower in old than in mature leaves (Table 1), suggesting that electron and carbon flow to isoprene emission increased less with increasing light intensity in old leaves, possibly explaining lower Q_{I90} in old leaves. However, given that Q_{A90} and Q_{J90} were similar among young and old leaves (Table 1), this suggestion does not explain lower Q_{I90} in old compared with young leaves. Regarding the control by S_{DMADP}, Q_{I90} actually decreased with increasing S_{DMADP} across leaves of different age, mainly reflecting the large S_{DMADP} in old leaves (Fig. 4a). As stated above, such a negative relationship is consistent with the feedback inhibition of MEP/DOXP

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pathway by DMADP pool size, i.e. light-dependent increase in S_{DAMDP} can be partly inhibited by its increasing concentration. Indeed, the light-dependent increase of S_{DAMDP} pool was greater in young and mature leaves than in old leaves (Table 1), and this increase was strongly correlated with Q_{I90} (Fig. 4c). Such a greater increase might reflect gradual DMADP-saturation of alternative DMADP-consuming reactions with increasing light level. Thus, age-dependent changes in saturation light are overall consistent with the hypothesis of a greater share of DMADP use by alternative DMADP sinks such as photosynthetic pigment synthesis in young and mature leaves relative to old leaves.

On the other hand, Q_{I90} scaled positively with the isoprene synthase rate constant (Fig. 4b) and the emission capacity (Fig. 5b) across leaves of different age. While these correlations are not necessarily causal, a greater isoprene synthase activity relative to DMADP pool size allows for enhanced consumption of DMADP as soon as it becomes available with increasing quantum flux density. Such a greater capacity works against accumulation of DMADP and associated potential feedback-inhibition of emission at higher light intensities, although it still did not avoid excessive DMADP accumulation in old leaves in our study.

Conclusions

We have demonstrated that the initial quantum yield and the saturation light of isoprene emission importantly vary in leaves of different age and that these modifications are primarily triggered by changes in the pool size of DMADP, the substrate for isoprene synthase. To our knowledge, such age-dependent differences in the shape of the light response curve driven by variations in substrate availability have not been reported before. In highly dynamic canopies supporting foliage of different age, modifications in the shape of the light response curves of isoprene emission clearly importantly alter the whole canopy isoprene emission rate. Thus, we argue that instead of using constant emission algorithms as widely employed in the trace gas exchange community (s. Introduction), age-dependent modifications need to be taken into account in simulating canopy isoprene emissions. In the simplest manner, age-dependent changes in the light response can be incorporated in models using empirical relationships between leaf age and quantum yield for isoprene emission.

Furthermore, analysis of past evidence indicates that differences in the light response of isoprene emission due to changes in DMADP pool size can be extended to plants grown under different atmospheric [CO_2] (Sun *et al.* 2012b) and under different temperatures (Rasulov *et al.* 2015a), suggesting that the substrate-level control of the shape of the light response is a general mechanism responsible for variations in the initial quantum yields. A mechanistic consideration of such changes is clearly difficult, because it would require information about DMADP pool size, which the models currently cannot predict. However, consideration of DMADP pool as an outcome of the competition by prenyltransferases and isoprene synthase might provide a promising opportunity, especially when an estimate of pigment content and turnover could be obtained by simple alternative techniques such as remote sensing (Lichtenthaler *et al.* 1996; Gamon & Surfus 1999; Sims & Gamon 2002; Peñuelas *et al.* 2013). We conclude that models linking all DMADP consuming processes, in particular, photosynthetic pigment synthesis and isoprene emission, could ultimately provide a way for fully mechanistic modeling of environmental responses of isoprene emission.

Although changes in the DMADP pool size provided an explanation for modifications in the initial quantum yield for isoprene emission, future research is needed to explain the mismatch between the capacities of plastidic MEP/DOXP pathway and isoprene emission.

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Summary statement

Isoprene emission rate under light-limited conditions is driven by the availability of the end-product of plastidic isoprenoid synthesis pathway, dimethylallyl diphosphate (DMADP). Apart from isoprene emission, prenyltransferase reactions leading to synthesis of photosynthetic pigments can reduce DMADP pool size, but the implications of such a possible competition on the isoprene emission light response were unknown. We demonstrated that DMADP available for isoprene synthesis increased through leaf development and aging in aspen, and this was associated with increases in the quantum yield and reduced saturation light of isoprene emission, indicating that age-dependent changes in DMADP consumption by competing sinks importantly alter the light response of isoprene release.

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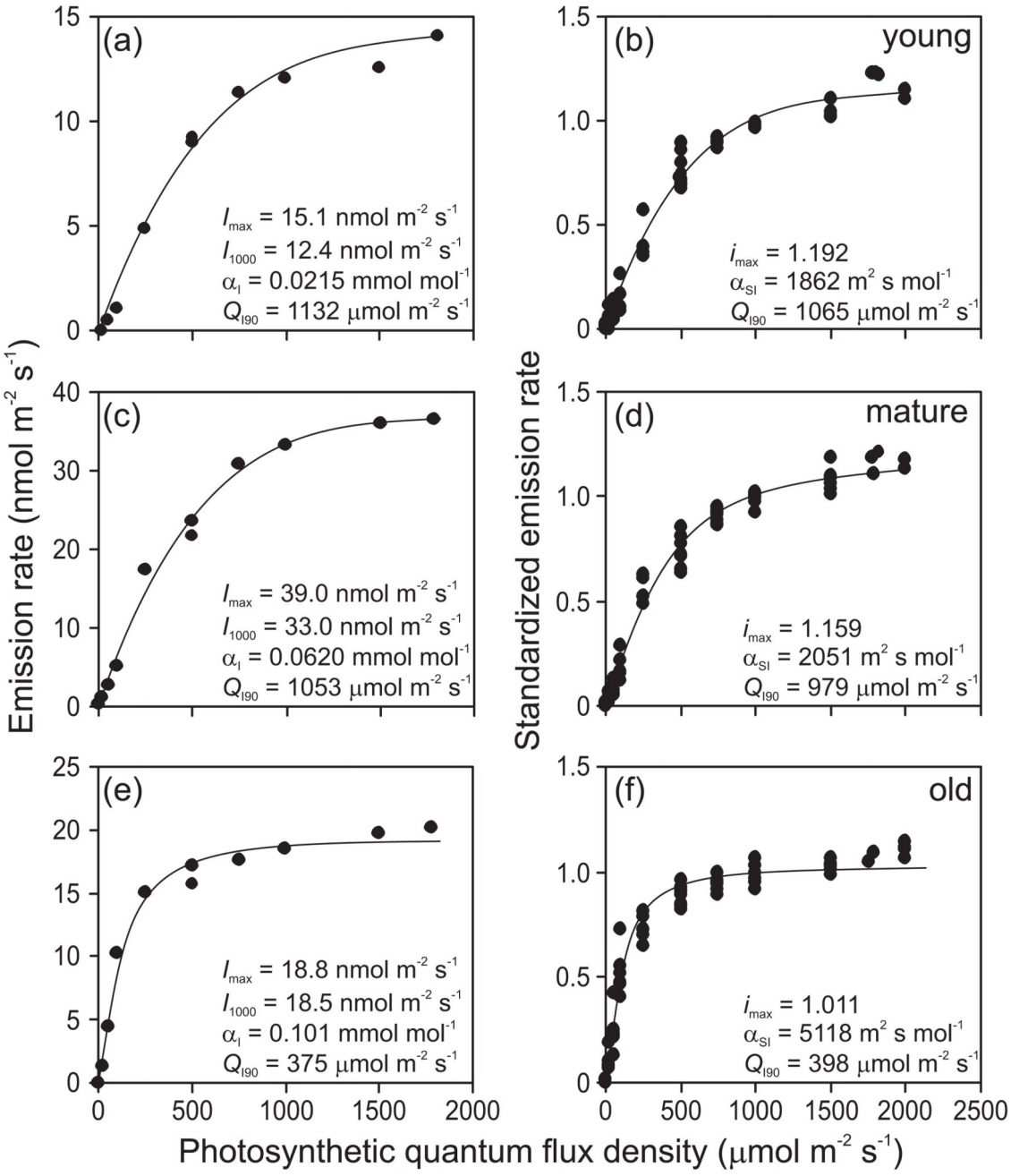

Fig. 1.

Representative light (*Q*) dependencies of isoprene emission rate for young (a), mature (c) and old non-senescent (e) leaves and comparison of standardized light dependencies of isoprene emission among young (b), mature (d) and old non-senescent (f) leaves of hybrid aspen (*Populus tremula* x *P. tremuloides*). Data in (a), (c) and (e) were fitted by Eq. 2 (r^2 = 0.989 for the young, r^2 = 0.991 for the mature and r^2 = 0.982 for the old leaf) with the parameters defined as: I_{max} - the isoprene emission capacity, α_I - the initial quantum yield of isoprene emission. In addition, the predicted isoprene emission rate at Q = 1000 μmol m^{-2}

s^{-1}, I_{1000}, and the value of Q that is necessary to achieve 90% of the process rate at the quantum flux density of 2000 μmol m^{-2} s^{-1}, Q_{I90}, (Eq. 3, saturating quantum flux density) are also shown. In (b), (d), and (f) all data (6 light response curves for each leaf age class pooled) were standardized with respect to I_{1000} and Eq. 2 was fitted to the data again (r^2 = 0.981 for young, r^2 = 0.983 for mature and r^2 = 0.968 for old leaves). Standardized response curve parameters are denoted as i_{max} (apparent capacity) and α_{SI} (apparent quantum yield). Young leaves (4th-5th from the apex) were ca. 12 days old, young fully mature leaves (10th-12th from the apex) were ca. 30 days old and old non-senescent fully mature leaves (18th-20th from the apex) were ca. 60 days old.

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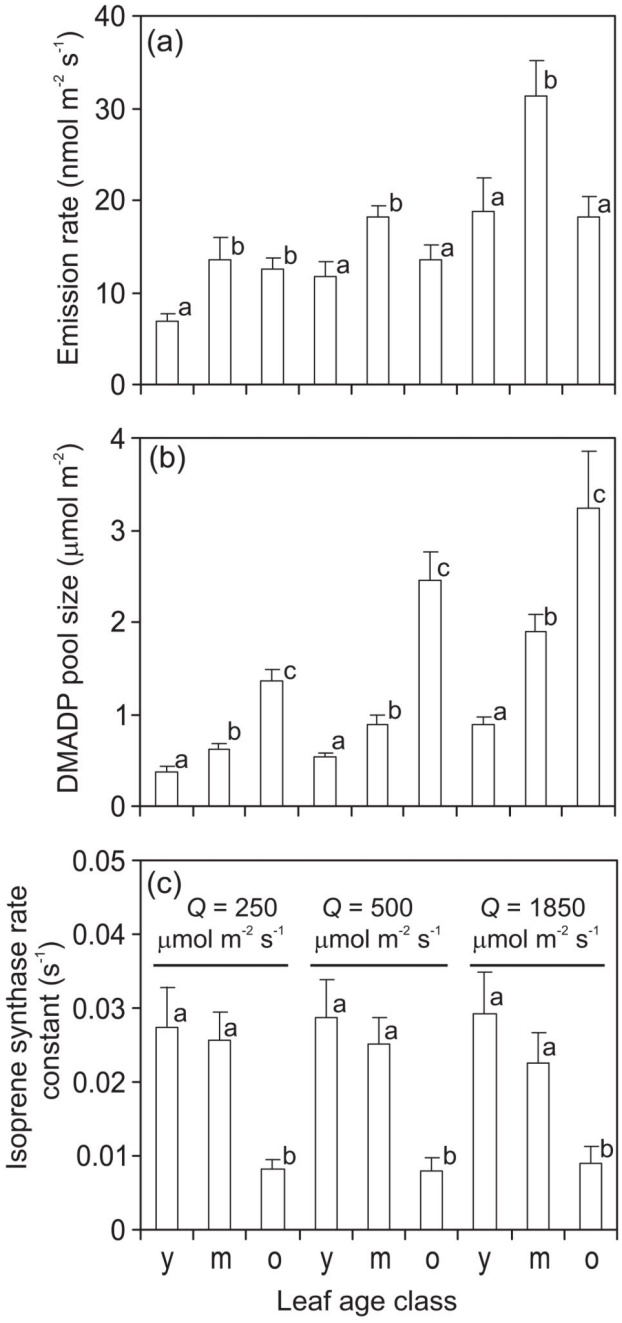

Fig. 2.

Leaf age dependent changes in isoprene emission rate (a), dimethylallyl diphosphate (DMADP) pool size (b) and in vivo isoprene synthase rate constant (c) at three different quantum flux densities (*Q*) in hybrid aspen (*P. tremula* x *P. tremuloides*) leaves. DMADP pool responsible for isoprene emission was measured from the postillumination release of isoprene emission (Rasulov *et al.* 2009a; Rasulov *et al.* 2010; Li *et al.* 2011), and the isoprene rate constant was taken as the initial slope of isoprene emission vs. DMADP pool size (Rasulov *et al.* 2011; Rasulov *et al.* 2014). Data are averages +SE ($n = 6$). Leaf age class

as: y = young, m = mature, o = old (Fig. 1 for a detailed description of leaf age classes). Averages with the same lowercase letter at given light level are not significantly different among leaf age classes according to paired samples *t*-tests ($P > 0.05$).

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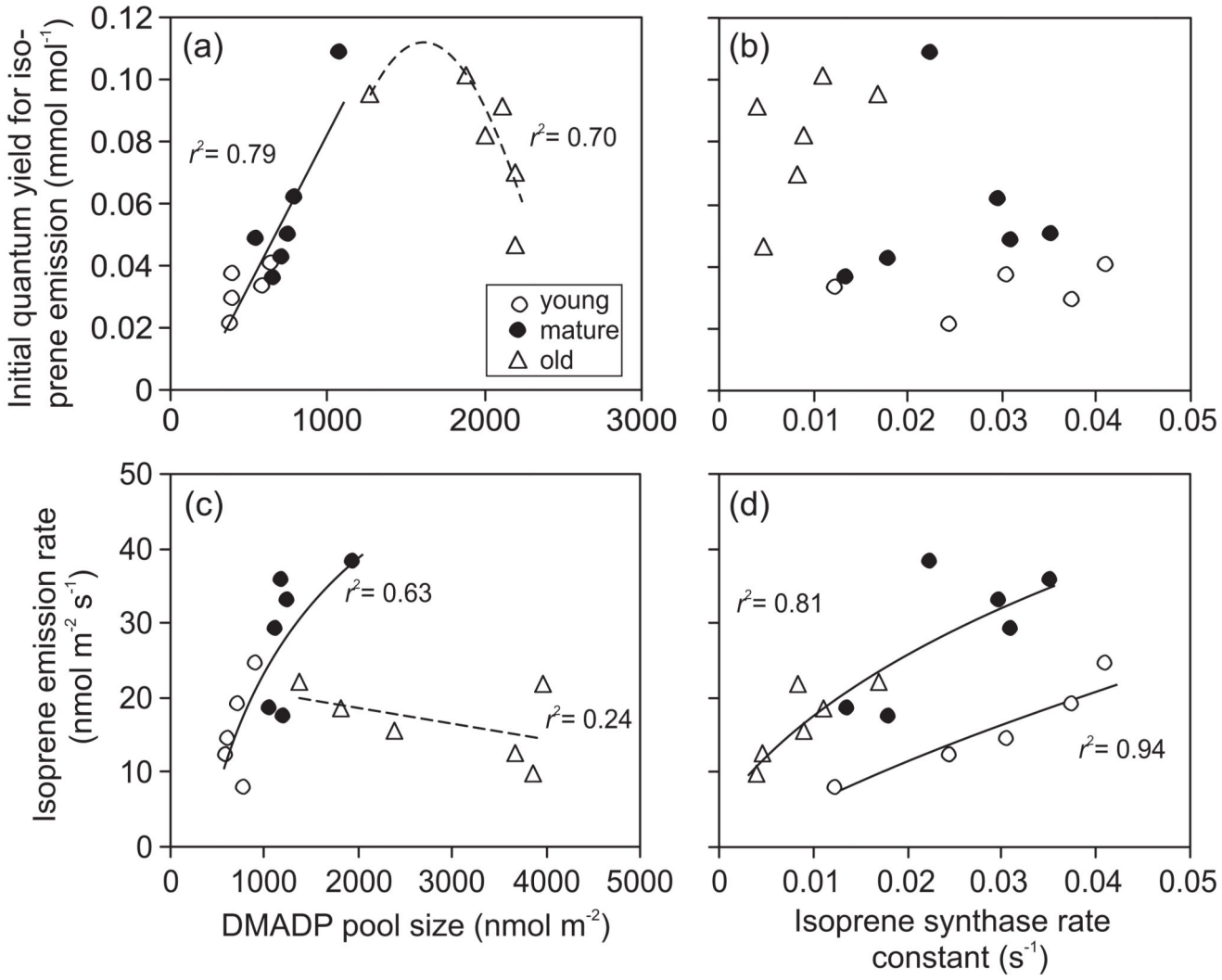

Fig. 3.

Correlations among the initial quantum yield of isoprene emission [α_I in Eq. 2; (a, b)], and isoprene emission rate at a high light of Q = 1000 μmol m^{-2} s^{-1} (c, d) with DMADP pool size (a, c) and isoprene synthase rate constant (b, d) in different-aged leaves of hybrid aspen (*P. tremula* x *P. tremuloides*). Estimation of DMADP pool size (S_{DMADP}) and isoprene synthase rate constant (k) as in Fig. 2. In (a), an average S_{DMADP} for Q values of 250 and 500 μmol m^{-2} s^{-1} was used, while in (b), an average S_{DMADP} for Q values of 500 and 1850 μmol m^{-2} s^{-1} was used. In (b) and (d), k is an average for all three light intensities because k did not vary with Q (Fig. 2). Data were fitted by linear and non-linear regressions ($P < 0.01$ for all regressions shown by solid lines, $P < 0.1$ for the regression shown by a dotted line in (a) and $P > 0.2$ for the regression shown by a dotted line in (c). In the regressions in (a) and (c), young and mature leaves were pooled, while in (d), old and mature leaves were pooled.

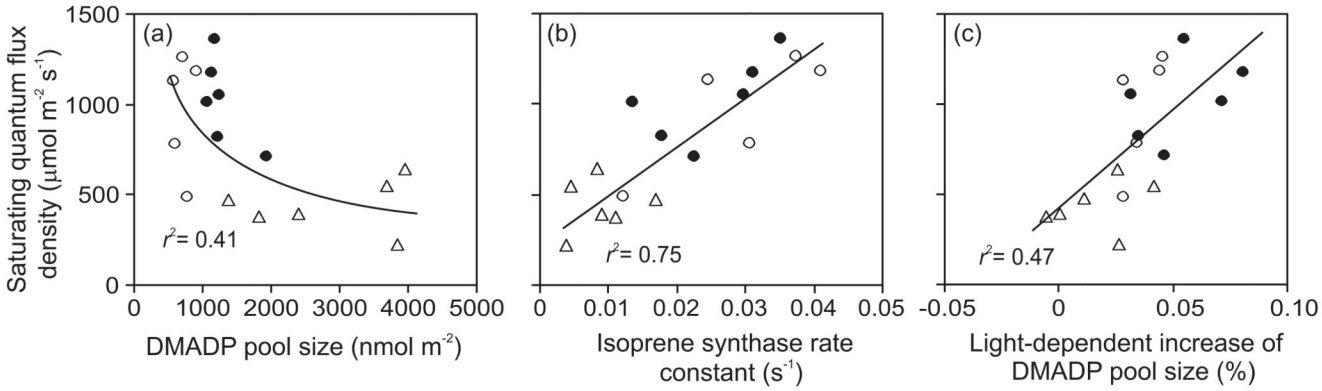

Fig. 4.

Correlations of saturating quantum flux density for isoprene emission (Q_{l90}, Eq. 3) with DMADP pool size (a), isoprene synthase rate constant (b) and normalized light-dependent increase of DMADP pool size [Δ_S, Eq. 5; (c)] in hybrid aspen (*P. tremula* x *P. tremuloides*) young, mature and old leaves (symbols as in Fig. 3, and definition of leaf ages as in Fig. 1). The saturating quantum flux density is defined as the value of Q required to reach 90% of the emission rate at Q = 2000 μmol m^{-2} s^{-1}. All data pooled were fitted by non-linear (a) and linear (b, c) regressions ($P < 0.01$). In (a), DMADP pool size is calculated as the average of measurements at high light intensities of 500 and 1850 μmol m^{-2} s^{-1}, while in (b), the rate constant is the average value for all the three light intensities (250, 500 and 1850 μmol m^{-2} s^{-1}) used for the measurements (Fig. 2 for the definitions and average values at different light intensities). Δ_S is calculated for light intensities of 500 (Q_1) and 1850 (Q_2) μmol m^{-2} s^{-1} (Eq. 5).

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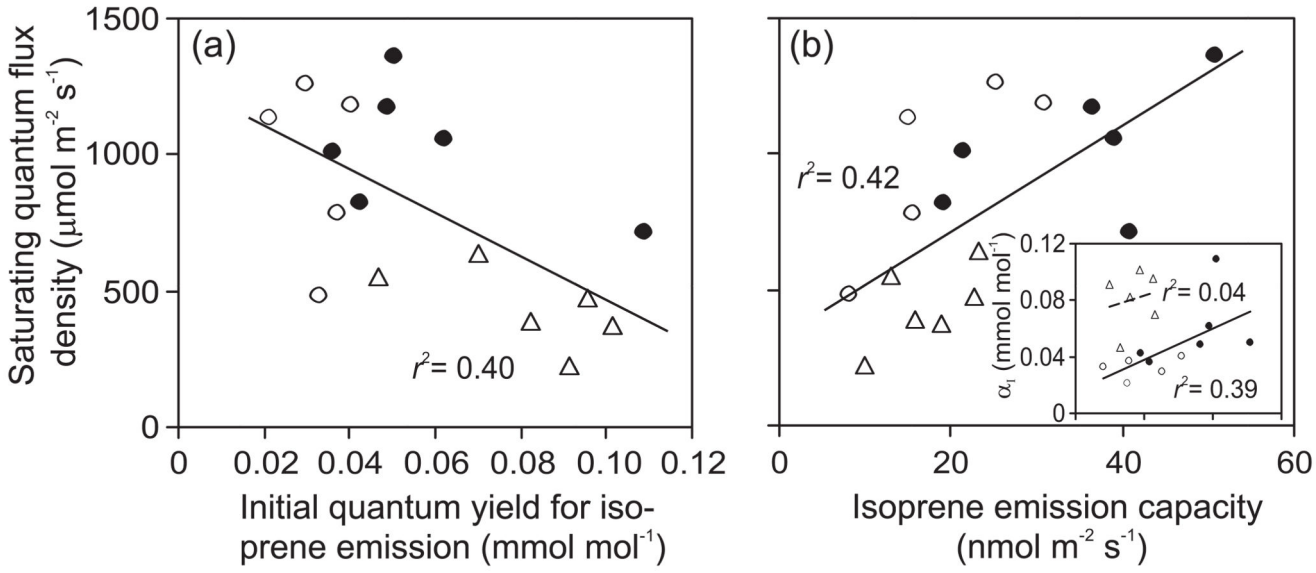

Fig. 5.

Relationships of saturating quantum flux density for isoprene emission (Eq. 3) vs. (a) the initial quantum yield of isoprene emission [α_I, (a)] and vs. the emission capacity [I_{max}, (b)], and the relationship of α_I vs. I_{max} [inset in (b)] in different-aged leaves of hybrid aspen (*P. tremula* x *P. tremuloides*). The Smith model parameters α_I and I_{max} are defined by Eq. 2. In the main panels, all data pooled (symbols as in Fig. 3 and leaf age classes as in Fig. 1) were fitted by linear regressions ($P < 0.01$). In the inset, separate fits were used for young and mature leaves pooled (solid lines, $P < 0.05$) and for old leaves (dashed line, $P > 0.9$).

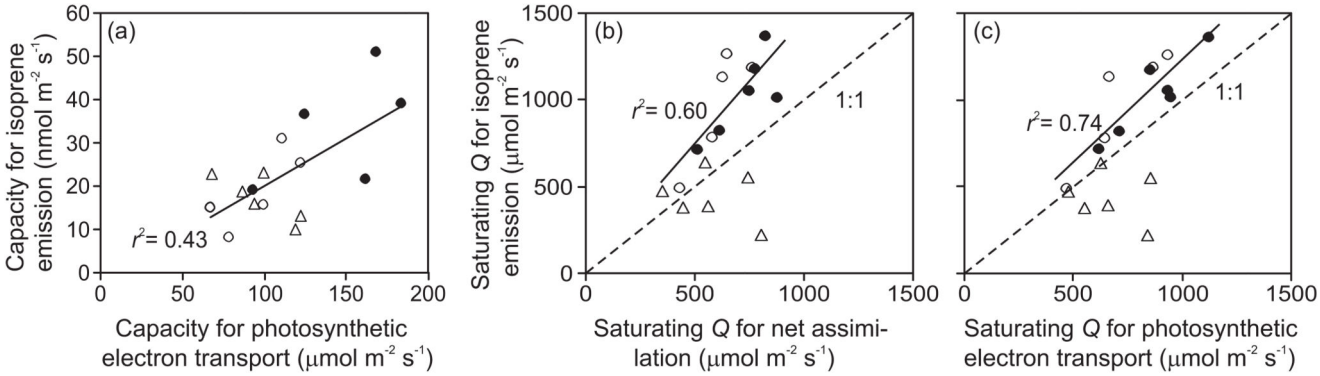

Fig. 6.

Correlations between the capacities for isoprene emission (I_{max}) and photosynthetic electron transport [J_{max}, (a)], and saturating quantum flux density (Q_{90}) for isoprene emission in relation to Q_{90} for net assimilation (b) and photosynthetic electron transport (c) and in different-aged leaves of hybrid aspen (*P. tremula* x *P. tremuloides*). The process capacities are defined by Eq. 2 and the saturating quantum flux density by Eq. 3. Data for all leaf ages pooled in (a), and data for young and mature leaves pooled in (b) and (c) were fitted by linear regressions (symbols as in Fig. 3 and leaf age classes as in Fig. 1, all regressions are significant at $P < 0.01$). Dashed lines in (b) and (c) denote the 1:1 relationships.

Table 1

Average (± SE) values of structural and isoprene emission and photosynthetic characteristics in different-aged leaves of hybrid aspen (*Populus tremula* x *P. tremuloides*)

Trait[1]	Young	Mature	Old
Structural traits			
Leaf area (cm^2)	48.7 ± 3.4a	54.7 ± 4.4a	48.8 ± 4.2a
Dry mass per unit area ($g\ m^{-2}$)	48 ± 7a	65.2 ± 3.6b	61.9 ± 1.5b
Dry to fresh mass ratio ($g\ g^{-1}$)	0.277 ± 0.018a	0.298 ± 0.018a	0.270 ± 0.013a
Isoprene emission traits			
I_{1000} ($nmol\ m^{-2}\ s^{-1}$)	15.7 ± 2.9a	28.7 ± 3.6b	16.8 ± 2.0a
I_{max} ($nmol\ m^{-2}\ s^{-1}$)	19.0 ± 4.0a	34.6 ± 5.0b	17.3 ± 2.2a
I_{max}/I_{1000}	1.18 ± 0.05a	1.20 ± 0.05a	1.027 ± 0.007b
α_I ($mmol\ mol^{-1}$)	0.0323 ± 0.0033a	0.0581 ± 0.011b	0.081 ± 0.008c
Q_{I90} ($\mu mol\ m^{-2}\ s^{-1}$)	970 ± 150a	1020 ± 100a	440 ± 60b
Assimilation traits			
A_{1000} ($\mu mol\ m^{-2}\ s^{-1}$)	9.2 ± 1.1a	14.0 ± 1.5b	11.0 ± 1.5ab
A_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	11.6 ± 1.1a	16.2 ± 1.7b	12.3 ± 1.5a
A_{max}/A_{1000}	1.30 ± 0.08a	1.192 ± 0.041ab	1.129 ± 0.028b
R_d ($\mu mol\ m^{-2}\ s^{-1}$)	1.90 ± 0.47a	1.14 ± 0.27ab	0.66 ± 0.25b
C_i ($\mu mol\ mol^{-1}$)	230 ± 15a	216 ± 16a	233 ± 16a
(Λ_S (%)	33.3 ± 5.6a	35.1 ± 4.7a	28.0 ± 4.4a
α_A ($mol\ mol^{-1}$)	0.0392 ± 0.0032a	0.04031 ± 0.0019a	0.04355 ± 0.0017a
Q_{A90} ($\mu mol\ m^{-2}\ s^{-1}$)	650 ± 60ab	730 ± 60b	580 ± 70a
Photosynthetic electron transport traits			
J_{1000} ($\mu mol\ m^{-2}\ s^{-1}$)	88 ± 8a	121 ± 13b	92 ± 7a
J_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	96 ± 10a	136 ± 17b	98 ± 8a
J_{max}/J_{1000}	1.076 ± 0.019ab	1.118 ± 0.025b	1.064 ± 0.013a
α_J ($mol\ mol^{-1}$)	0.257 ± 0.025a	0.279 ± 0.016a	0.282 ± 0.009a
Q_{J90} ($\mu mol\ m^{-2}\ s^{-1}$)	720 ± 80ab	870 ± 70b	670 ± 60a
Combined traits			
I_{max}/A_{max} ($mmol\ mol^{-1}$)	1.77 ± 0.35a	2.13 ± 0.38a	1.63 ± 0.40a
I_{max}/J_{max} ($mmol\ mol^{-1}$)	0.194 ± 0.030a	0.228 ± 0.031a	0.191 ± 0.037a
α_I/α_A ($mmol\ mol^{-1}$)	0.84 ± 0.09a	1.20 ± 0.12b	1.88 ± 0.21c
α_I/α_J ($mmol\ mol^{-1}$)	0.128 ± 0.013a	0.173 ± 0.014b	0.288 ± 0.028c
Q_{I90}/Q_{A90}	1.56 ± 0.15a	1.41 ± 0.07a	0.85 ± 0.16b
Q_{I90}/Q_{J90}	1.33 ± 0.11a	1.181 ± 0.044a	0.70 ± 0.11b

Average values with the same lowercase letter are not significantly different ($P > 0.05$ according to separate samples *t*-tests)

[1]Isoprene emission traits: I_{1000} - rate at a quantum flux density (Q) of 1000 μmol m^{-2} s^{-1}, I_{max} - emission capacity (Eq. 2), α_I - initial quantum (Eq. 2), Q_{I90} - Q for 90% of I at Q = 2000 μmol m^{-2} s^{-1} (saturation Q, Eq. 3); net assimilation traits: A_{1000} - rate at a quantum flux density (Q) of 1000 μmol m^{-2} s^{-1}, A_{max} - gross photosynthetic capacity (Eq. 2), R_d - dark respiration rate, C_i - average intercellular CO_2 concentration for Q ≥ 200 μmol m^{-2} s^{-1}, Λ_S - relative stomatal limitation of photosynthesis at light saturation, α_A - initial quantum (Eq. 2), Q_{A90} - Q for 90% of A at Q = 2000 μmol m^{-2} s^{-1} (Eq. 4); photosynthetic electron transport traits: J_{1000} - rate at a quantum flux density (Q) of 1000 μmol m^{-2} s^{-1}, J_{max} - electron transport capacity (Eq. 2), α_J - initial quantum (Eq. 2), Q_{J90} - Q for 90% of J at Q = 2000 μmol m^{-2} s^{-1} (Eq. 3).